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TITLE: Determination of the Role of Estrogen Receptors and Estrogen Regulated Genes in B Cell Autoreactivity

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14. ABSTRACT

Systemic lupus erythematosus (SLE) is an autoimmune disease that occurs preferentially in women. In murine models of SLE, it is clear that increased or sustained high physiologic levels of estradiol can accelerate onset of disease and exacerbate disease severity. We have shown that estradiol alters B cell maturation in vivo but does so in a genetically restricted fashion. We have also shown that estradiol can act directly on B cells to alter B cell receptor (BCR) signalling strength.

This proposal is to understand which estrogen receptors mediate the effects of estradiol on B cell survival, maturation and activation in order to assess whether hormonal manipulation has a potential therapeutic role in SLE. The proposal is further designed to ask why estradiol affects B cell function in mice of one genetic background but not another.

15. SUBJECT TERMS
Systemic Lupus, Estrogen, BCR Signaling, B Cell Maturation, B Cell Selection

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Introduction:

Progress has been made in all aims of the proposal and two manuscripts have been submitted for publication. One is under revision.

Body:

1) Determine which estrogen receptor is responsible for estrogen-induced alterations in BCR signaling.

Our original observation was that estradiol expands the marginal zone subset and diminishes the strength of B cell receptor (BCR) signaling. We hypothesized that these two events were related and that the decrease in BCR signaling led to an expanded marginal zone. We decided to explore this in mice lacking estrogen receptor (ER) α or β to determine whether the same ER transduced both effects. We found the ERα sufficient mice displayed an estradiol induced expansion of MZ B cells and a decrease in BCR signaling.

We determined that ERα also was sufficient to cause the expansion and activation of autoreactive B cells. In contrast, ERβ engagement diminished B cell lymphopiesis, though not nearly to the same degree as ERα engagement, but did not lead to MZ B cell expansion or a change in BCR signal strength and autoreactivity. We performed these studies in ERα and β deficient mice and in wild type mice to which we administered ERα and ERβ specific agonists.

We believe these studies suggest that an ERα specific antagonist, delivered to B cells, might be an effective approach to therapy in SLE.

2) Analyze B cell maturation and selection in placebo or estrogen-treated C57Bl/6 mice.

We have previously seen that estradiol will induce increased titers of anti-DNA antibody in R4A transgenic BALB/c but not R4A transgenic C57Bl/6 mice. These strains harbor the same IgG26 anti-DNA heavy chain transgene with the same copy number and same chromosomal insertion site. Expression of estrogen receptors and metabolism of estradiol did not differ between the strains (Fig 1). Estradiol altered B cell maturation in the bone marrow in a similar fashion in both strains leading to fewer immature cells (Fig 2). B cell subsets in the spleen were also altered similarly by estradiol treatment in both strains (Fig 3). Interestingly, there was an increased in transgene-expressing B cells in

the spleens of C57Bl/6 mice compared to BALB/c mice (Fig 4) although estradiol increased this population in both strains.

Most importantly estradiol treatment rendered BALB/c transitional B cells less vulnerable to anti-IgM mediated apoptosis, but failed to have the some effect on C57Bl/6 transitional B cells. This was true for the T2 subset, but not the T1 (Fig 5) and was consistent with a reduced BCR-mediated calcium flux induced by estradiol in the BALB/c T2 subset but not the C57Bl/6 T2 subset (Fig 6). We are currently determining the molecular basis for this difference. It is clear that apoptotic pathways are altered by estradiol BALB/c B cells leading to a greater ratio of anti-apoptotic to proapotoptic molecules. We are continuing to explore the basis for the estradiol-induced altered BCR signaling.

3) Determine the role of antigen of estradiol-induced changes in B cell function.

We have now demonstrated that R4A transgenic mice given estradiol and DNase do not develop autoimmunity and do not display an expansion of anti-DNA B cells in either the transitional or mature subset (Table 1) DNase does not alter the expansion of transgene-expressing B cells, nor does it alter the estradiol-induced changes in B cell maturation (Fig 7). Interestingly, the presence of high affinity anti-DNA antibodies leads to increased BAFF expression which is abrogated in DNase treated mice (Fig 8). We have also shown that DNase given after estradiol administration protects glomeruli (Fig 9).

This suggests that DNase may affect antigen expression in the kidney as well as B cell maturation.

Key Research Accomplishments:

1**.** We have demonstrated that the maturational changes in B cells induced by estradiol are mediated through ERα.

2. We have demonstrated that ERα engagement also breaks B cell tolerance.

3. We have shown that estradiol alters B cell development in C57Bl/6 mice, but fails to alter BCR signalling

4. We have shown that the effects of estradiol require the presence of antigen.

Reportable outcomes:

Invitation to 2010 symposium on hormones and the immune system

Speaker at 2009 Neuroimmunology symposium on immune system and hormones

Two manuscripts in submitted

One manuscript in preparation

Conclusion:

We have now clearly shown that ERα is responsible for the estrogen-induced alterations in B cell maturation. It will, therefore, be important to test ERα antagonists in murine studies of B cell development and in murine models of lupus. This approach to therapy might provide clinical benefit without immunosuppression or intolerable masculinization in women.

The continued studies of BCR signaling and estrogen may identify other process that are critical in lupus pathogenesis and can be modulated by sex hormones. These studies may have implications for many diseases the phenotype of which is altered by hormone exposure. We have noted that recent study showing that exposure to estrogen increases risk of developing SLE.

References:

Cohen-Solal, J., Jeganathan, V., and Diamond, B. Estrogen-induced resistance to apoptosis in immature B cells is genetically determined: implications for Lupus (submitted)

Hill, L., Jeganathan, V., Chinnasamy, P., Grimaldi C., and Diamond, B. Role of estrogen receptor α in B cell maturation and selection (submitted)
Jeganathan, V. and Diamond, B. Long term effects on B cell repertoire of short term exposure to estrogen or prolactin (in preparation)

Appendices:

None

Supporting Data:

See attachments of Table and Figures

Table 1. Frequency of high affinity DNA-reactive B cells in R4A Tg mice treated with E2 and DNase

	PBS	E2	E2+DNase
Transitional	5/50 (10%)	12/52 (23%)*	6/63 (9.5%)
Mature	8/60 (13%)	18/62 (29%)*	10/72 (13.8%)

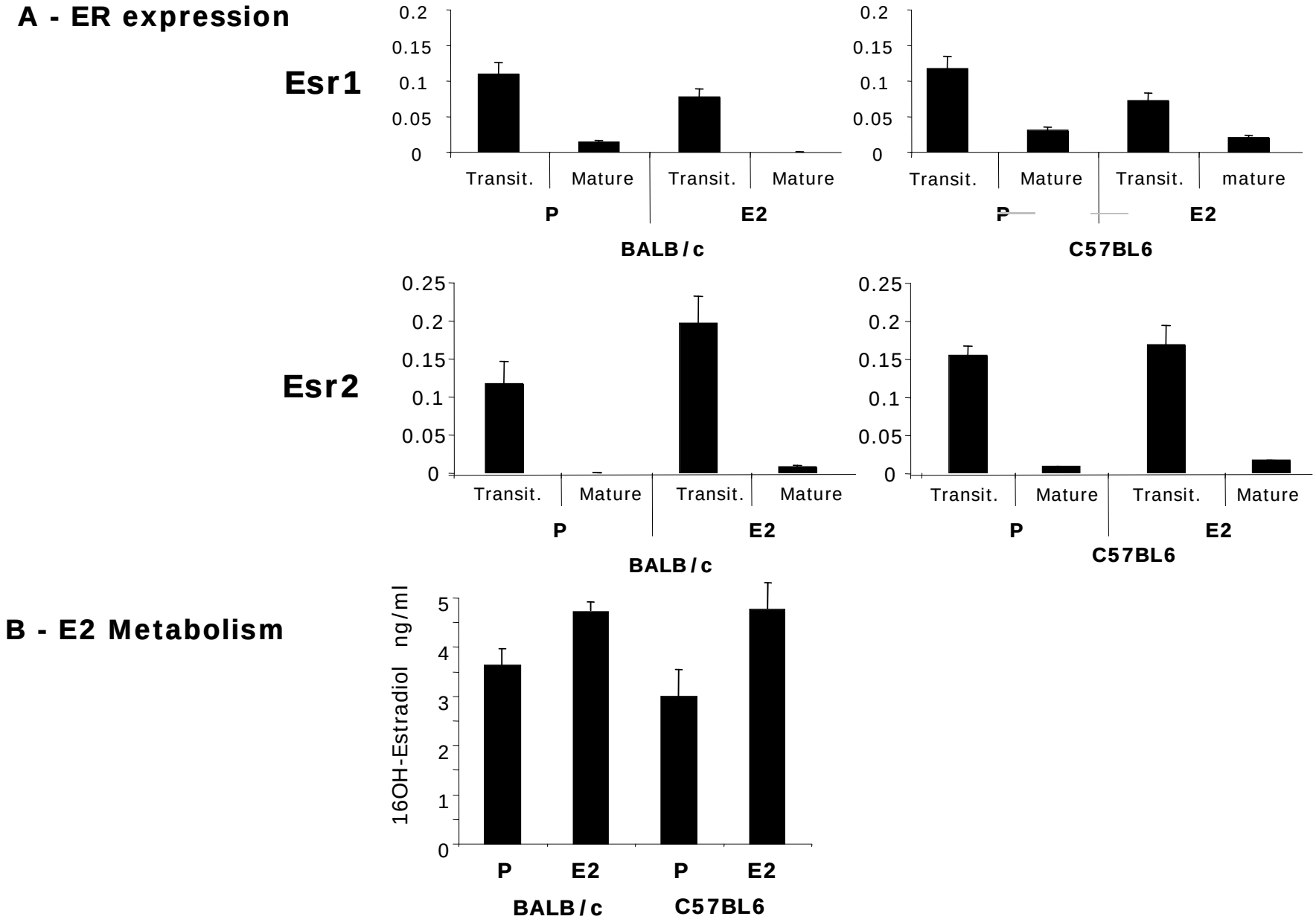


Figure 1: (A) expression of estrogen receptors ERalpha(Esr1) and ERbeta (Esr2) in splenic B cells and (B)Urinary 16 OH-Estradiol metabolite in BALB/c and C57BL6 mice.

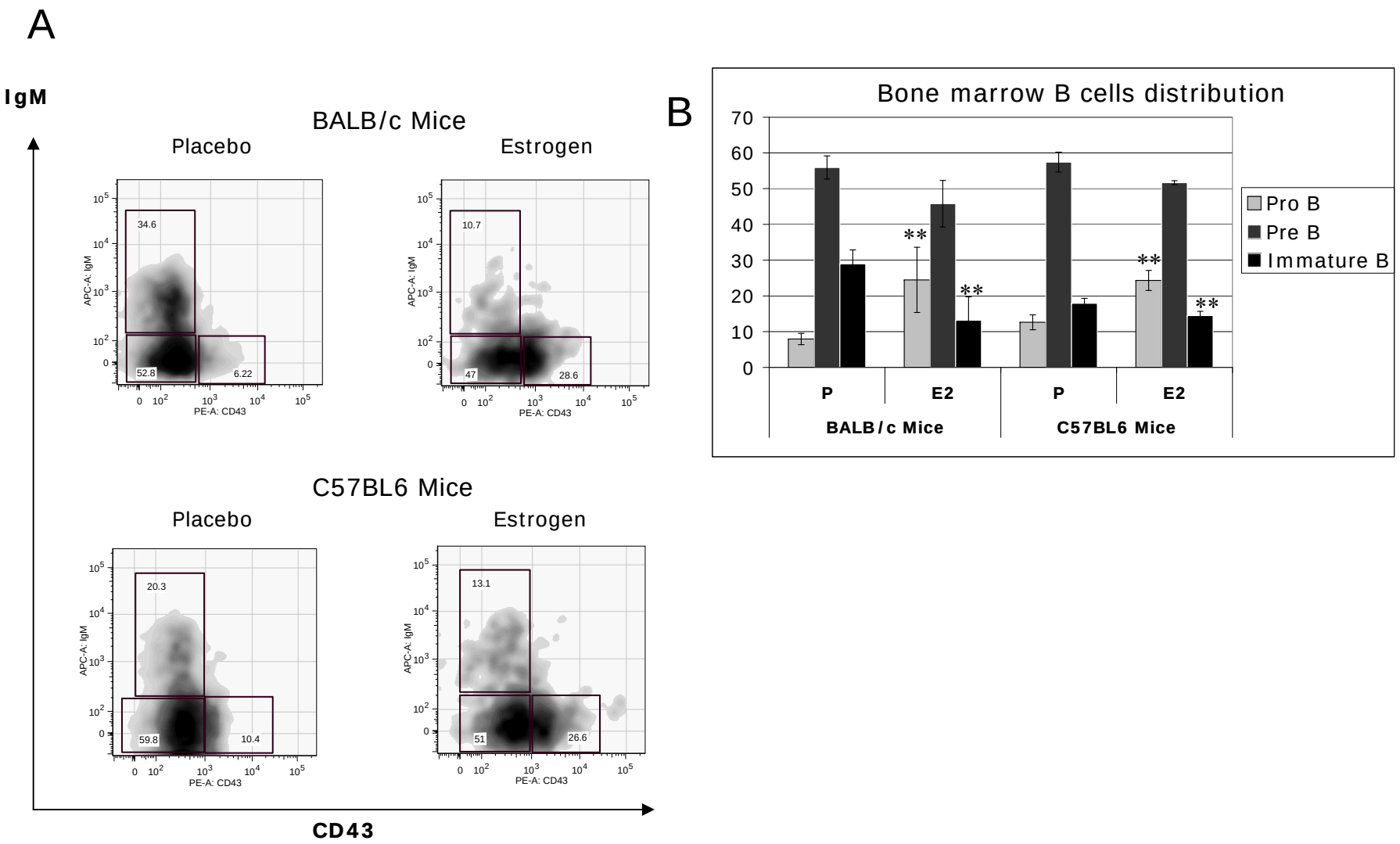


Figure 2 : Estadiol efforts on bone marrow B cells development in wild type BALB/c and C57BL/6 mice (A) Dot Plot of bone marrow cells gated on B220 intermediate cells and CD43 x IgM; (B) chart of bone marrow B cell distribution. Estradiol leads to maturation arrest in both strains (** $p \leq 0.01$).

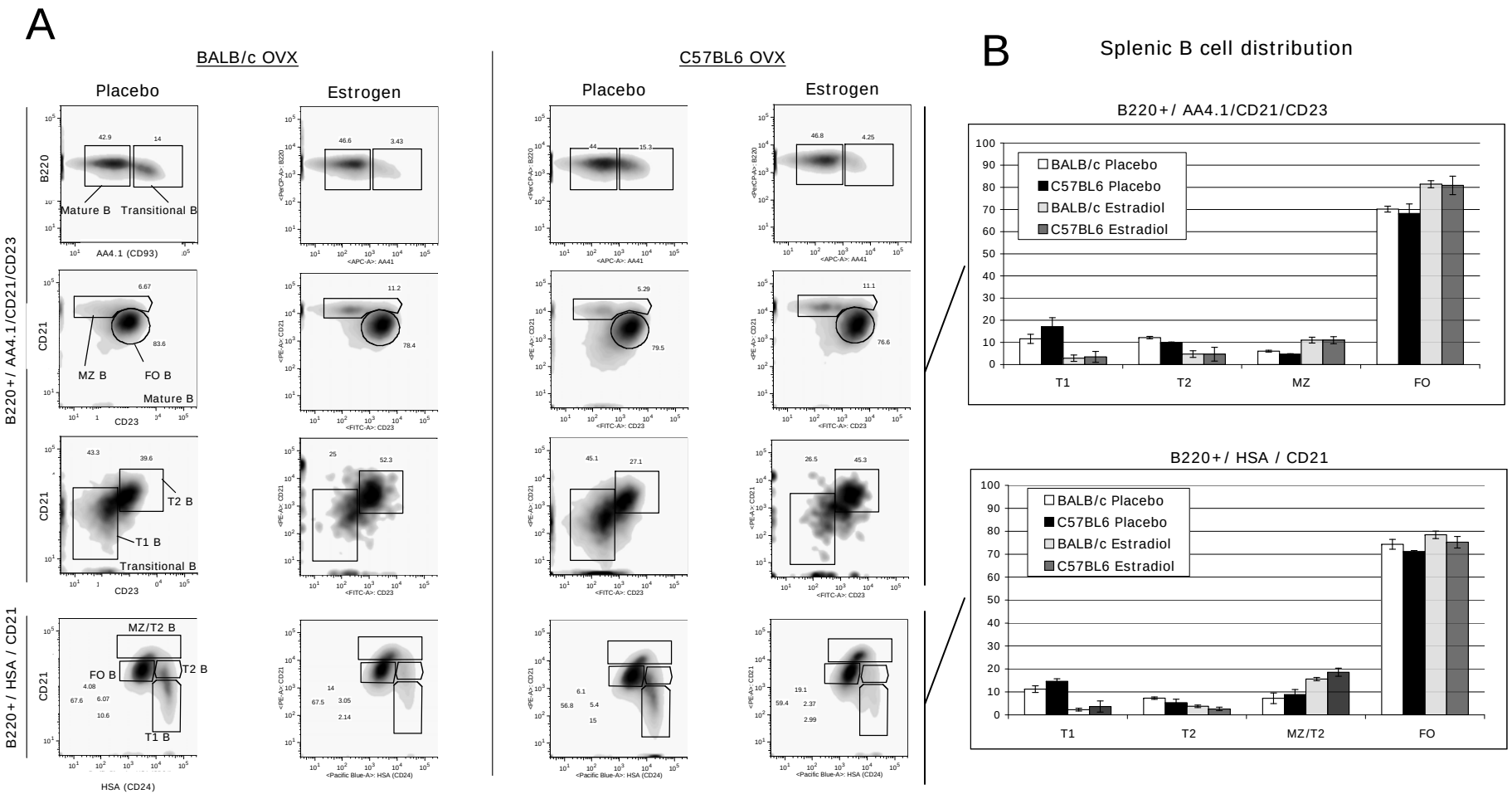
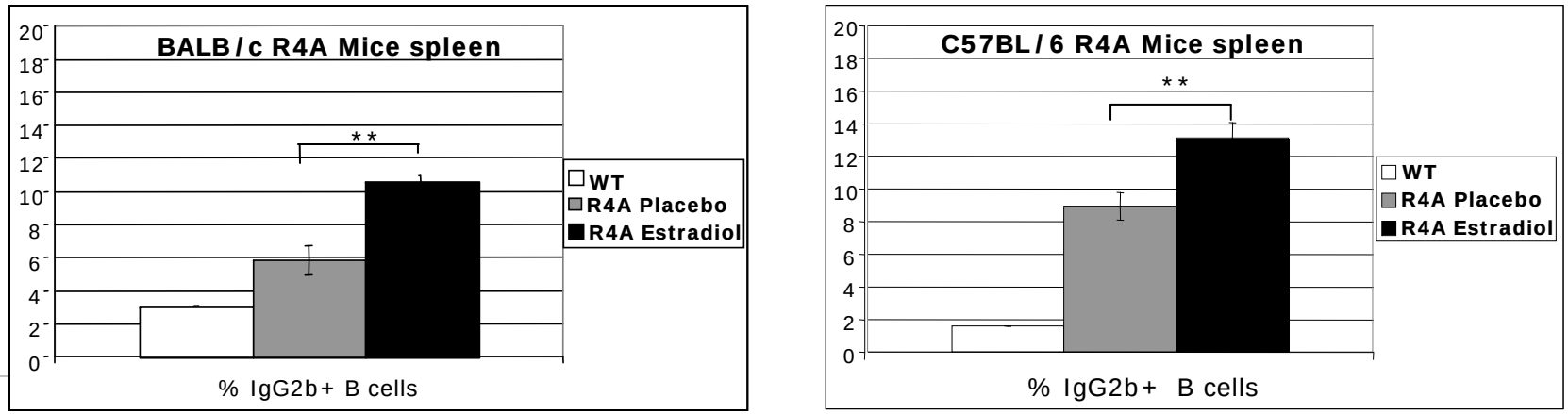


Figure 3 : Estrogen effects on splenic B cell development in wild type BALB/c and C57BL/6 ovariectomized (ovx) mice (A)Two strategies of gating to estimate B cell subtype distribution (AA4.1 and CD21, CD23 on B220+ cells versus HSA and CD21 on B220+ cells). (B) Chart of the splenic B cell distribution.

A



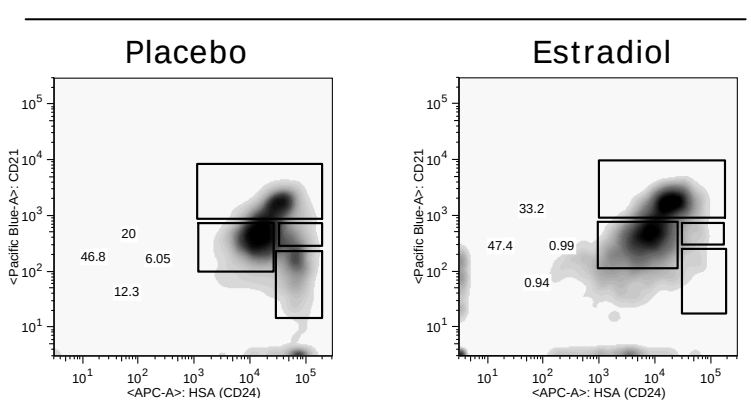
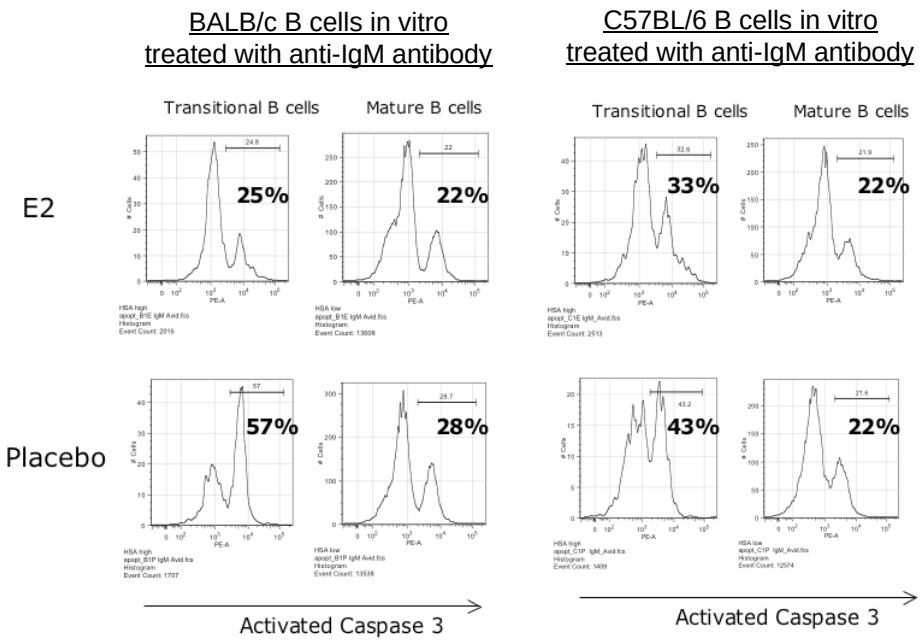


Figure 4 : Expansion of IgG2b (transgenic BCR R4A) in splenic B cells following estradiol treatment
(A) proportion of IgG2b+ (transgene expressing B cells) in splenic B cells (**p≤0.001)
(B) transitional and mature B cell distribution.

A



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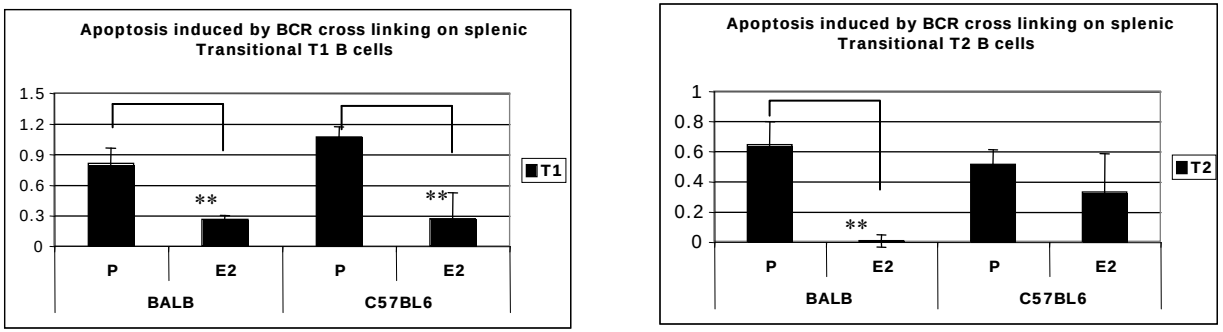

Figure 5 Apoptosis of splenic B cells triggered by BCR aggregation
(A) Transitional BALB/c B cells from estradiol treated mice are resistant to apoptosis while transitional C57BL/6 B cells are less resistant.
(B) T1 B cells of both strains are equally resistant to apoptosis while BALB/c T2 B cells are specifically resistant. (** $p<0.005$)

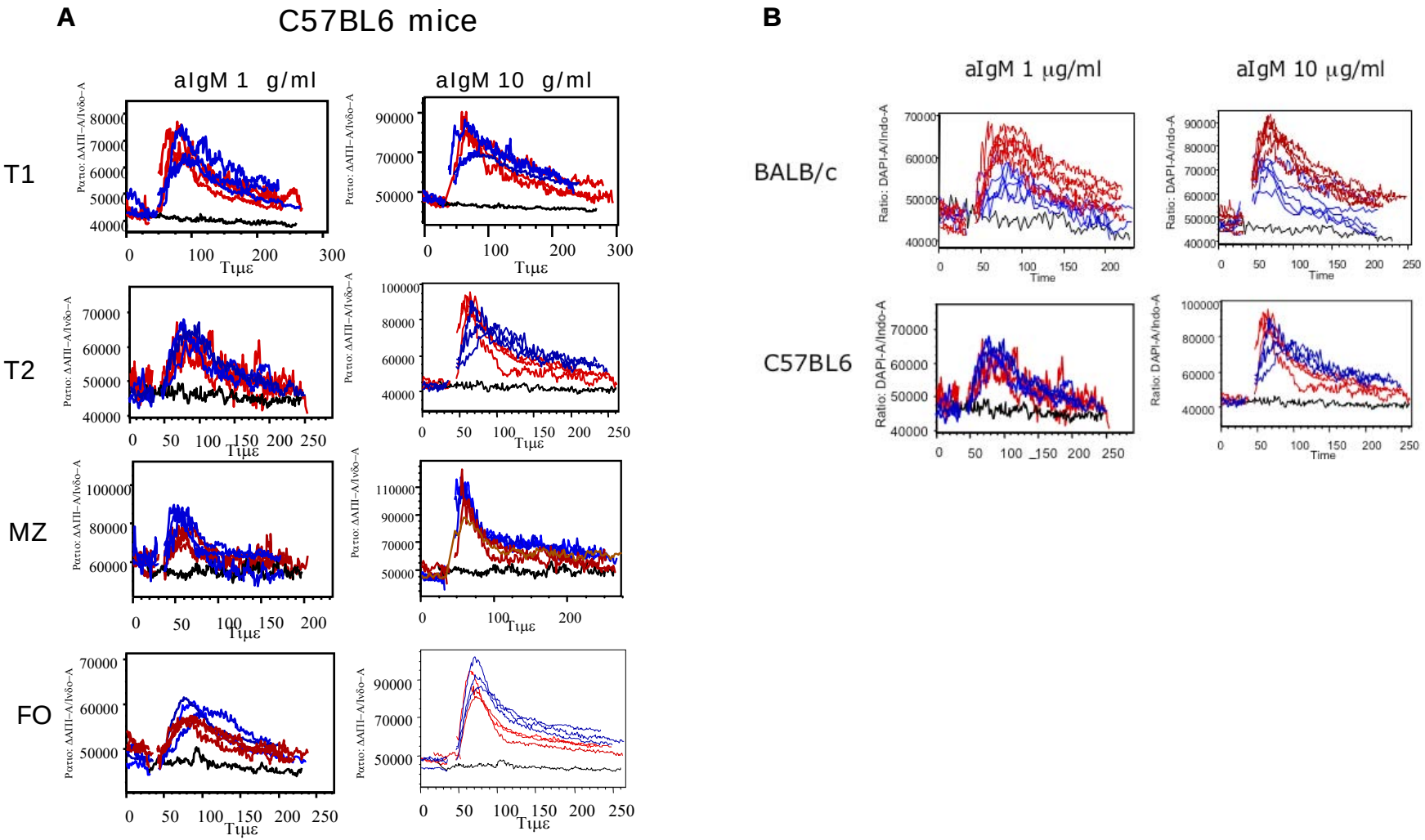

Figure 6 : Reduced calcium flux induced by estradiol treatment is strain specific
(A) Calcium flux triggered by BCR stimulation with increasing doses of anti IgM GaM Fab'2 on splenic B cells from ovariectomized C57BL6 mice treated 4 weeks with placebo (red) versus estradiol (blue) pellets.
(B) BALB/c T2 (B220pos AA4.1high CD23pos CD21pos) B cells from estradiol-treated mice, not C57BL6 T2 B cells, have a lower calcium spike upon non saturating BCR aggregation than B cells from placebo-treated mice.

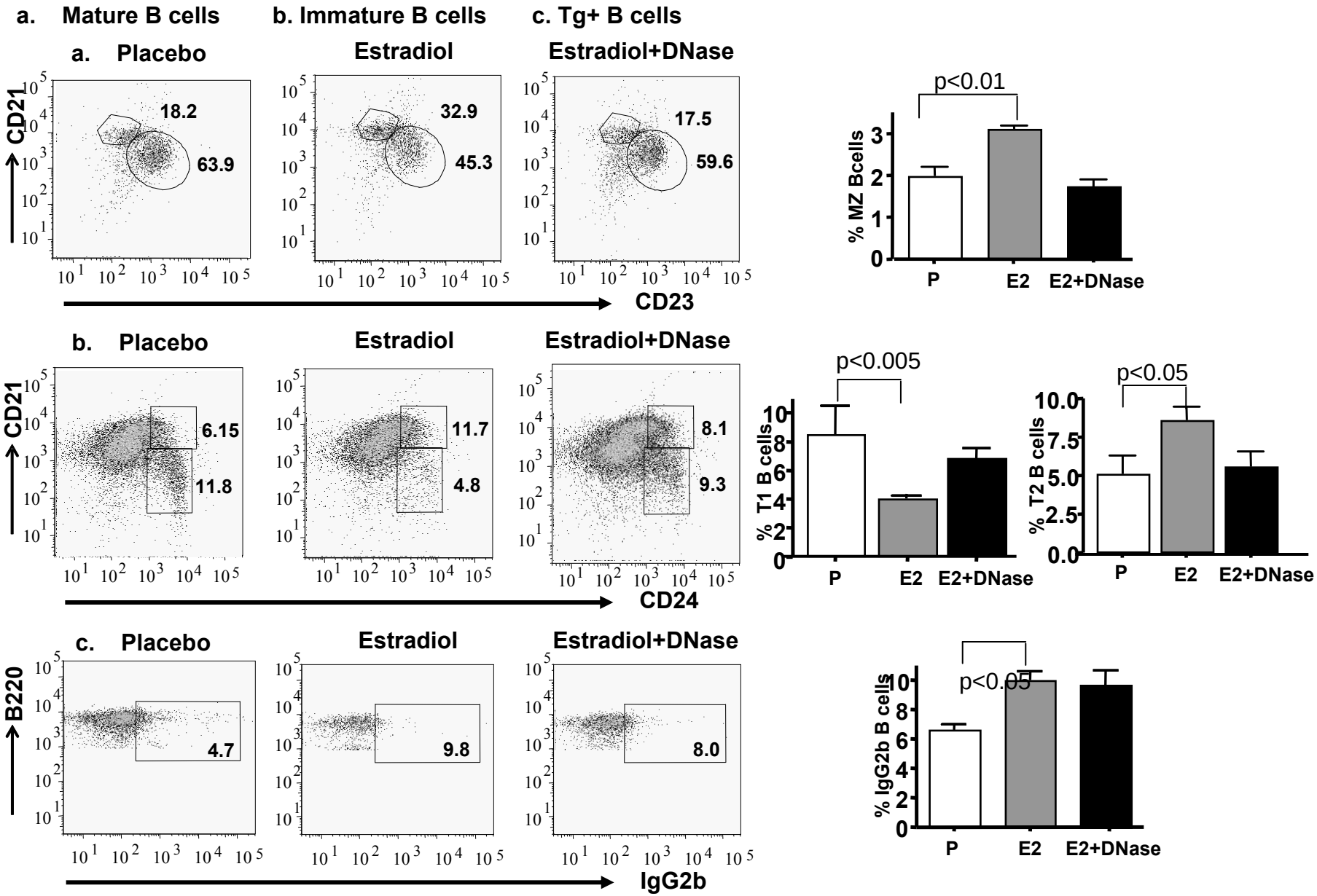

Figure 7: B cell subsets in R4Aγ2b BALB/c mice

B cell subsets are altered by exposure to estradiol, but normalized in mice given DNAse

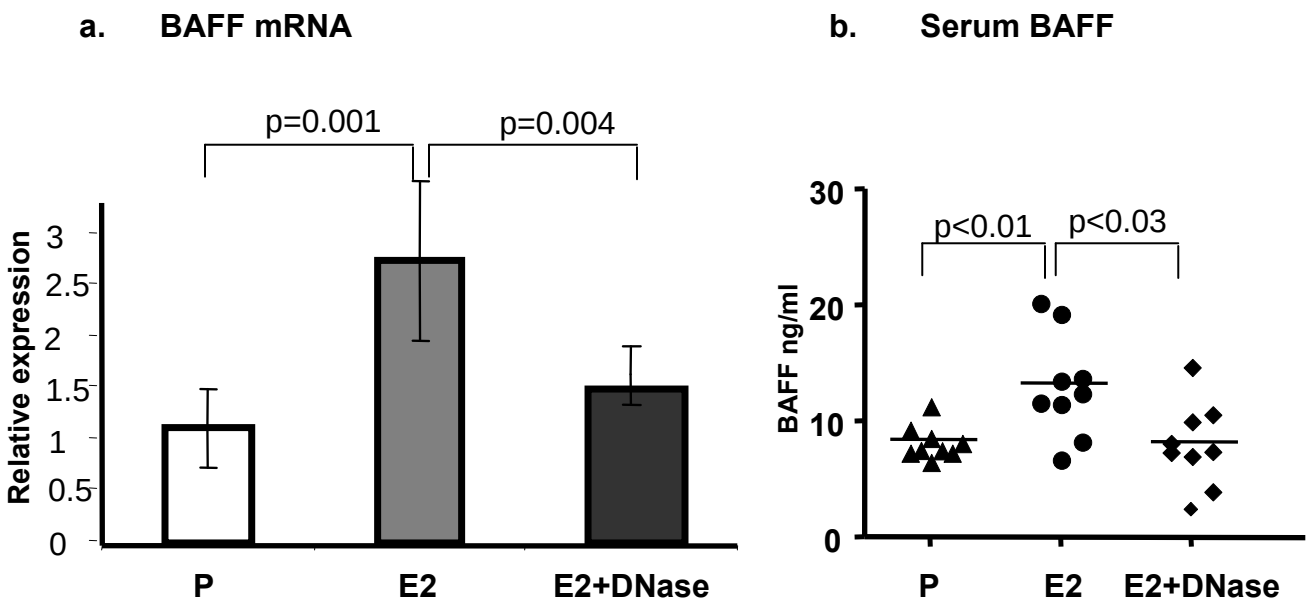

Figure 8: BAFF levels in R4Aγ2b BALB/c mice treated with placebo or estradiol with or without DNAse

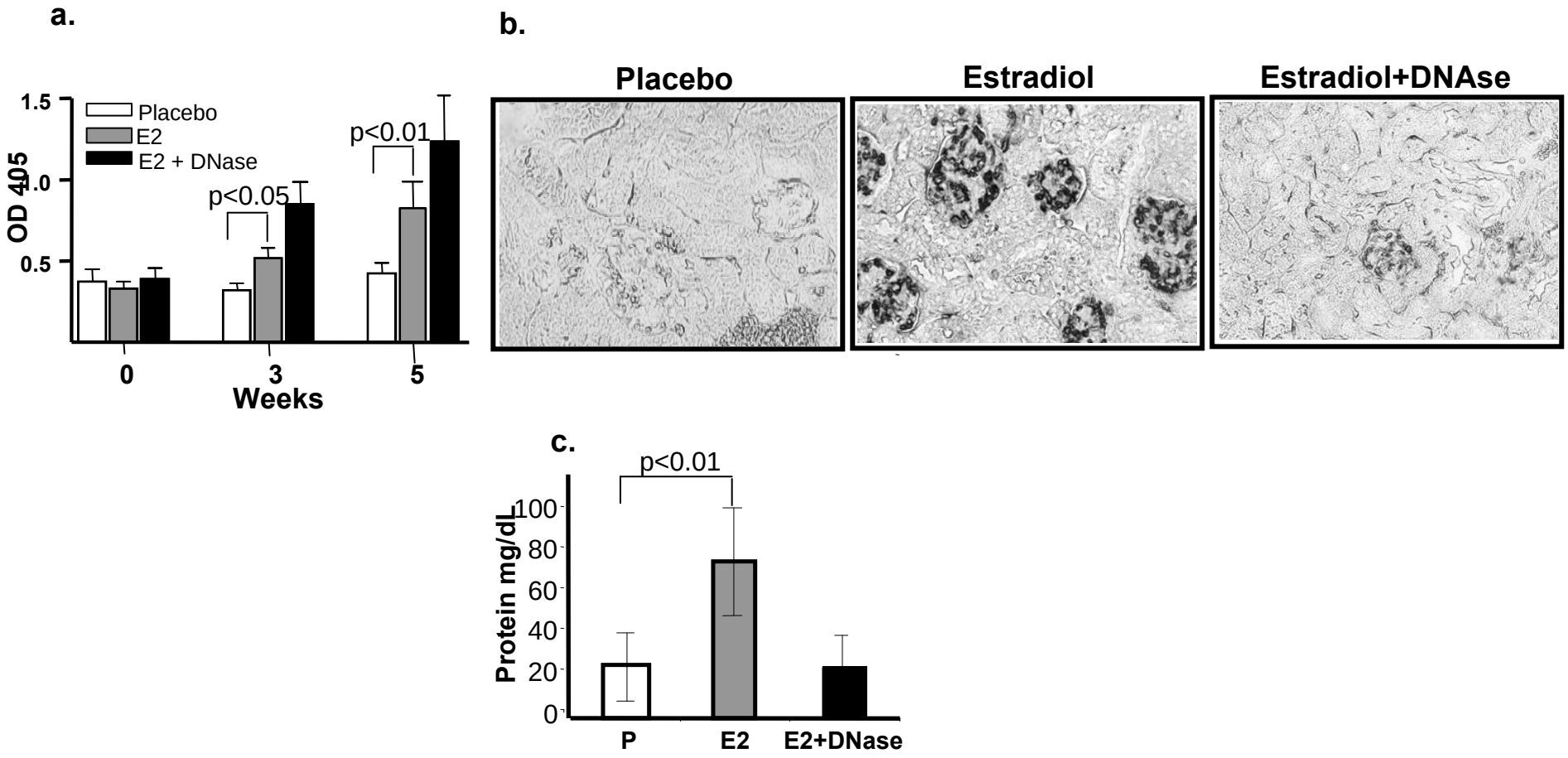

Figure 9: Serum anti-dsDNA antibodies (a), glomerular IgG deposition (b) and proteinuria (c) in R4Aγ2b BALB/c mice